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10/767,064	01/29/2004	Tony Peled	24024-506	5661
30623 7590 01/18/2007 MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C. ONE FINANCIAL CENTER BOSTON, MA 02111			EXAMINER SINGH, ANOOP KUMAR	
			ART UNIT 1632	PAPER NUMBER
SHORTENED STATUTORY PERIOD OF RESPONSE			MAIL DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary

Application No.

10/767,064

Applicant(s)

PELED ET AL.

Examiner

Anoop Singh

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 25 October 2006.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 201-219, 224-243 is/are pending in the application.
- 4a) Of the above claim(s) 202-208, 215-219, 224-237 and 240-243 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 201, 209-214, 238 and 239 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☒ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☒ None of:
- 1) ☒ Certified copies of the priority documents have been received.
 - 2) ☐ Certified copies of the priority documents have been received in Application No. _____.
 - 3) ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☒ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date 6/29/04; 9/22/06
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: _____

DETAILED ACTION

Applicant's amendment to the claims filed on October 25, 2006, has been received and entered. Claims 220-223 have been canceled, while claims 201 and 210 have been amended.

Claims 201-219, 224-243 are pending in the instant application.

Election/Restrictions

Applicants' election of claims 201, 209-215, 217-231, 235, 238 and 239 (Group I) in the reply filed on October 25 is acknowledged. Applicants have also elected culturing the cells in presence of one copper chelator (claims 201), neonatal umbilical cord cells (claim 209), FLT-3 ligand (claim 212) and granulocyte colony-stimulating factor (claim 214) as election of species for the elected invention. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 202-208, 216-218, 232-234, 236-237 and 240-243 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. In addition, Claims 215-219, 224-231 are also withdrawn since these claims are not directed to elected species of culturing cells in presence of at least one copper chelator. Election was made **without** traverse in the reply filed on October 25, 2006.

Claims 201, 209-214, 238 and 239 are under current examination.

Specification

The disclosure is objected to because of the following informalities: The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01. See page 41, line 4; page 45, line 31).

The year for reference of Itoh et al on page 45, last paragraph is not correct.

The specification has not been checked to the extent necessary to determine the presence of all possible minor error. Applicant's cooperation is requested in correcting errors of which applicant may become aware in the specification.

Appropriate correction is required.

Information Disclosure Statement

The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609.04(a) states, "the list may not be incorporated into the specification but must be submitted in a

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separate paper." Therefore, unless the references have been cited on PTO-1449 or by the examiner on form PTO-892, they have not been considered.

The information disclosure statement (IDS) submitted on 06/29/02 and 9/29/06 have been considered by the examiner.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 201, 209-214, 238 and 239 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of expanding an *ex vivo* population of CD34+ and Cd34+CD38- hematopoietic stem cell in culture, while at the same time inhibiting differentiation of the said cell *ex vivo* in culture medium; said method comprising:

(a) providing hematopoietic mononuclear cells that are not enriched prior to culturing , culturing said MNC *ex vivo* in culture under conditions allowing the proliferation and at the same time; said conditions for *ex-vivo* cell proliferation comprises providing either (i) early acting cytokines selected from the group consisting of stem cell factor, FLT3 ligand, interleukin-1, interleukin-2, interleukin-3, interleukin-6, interleukin-10, interleukin-12, tumor necrosis factor- α and thrombopoietin; and/or (ii) a late acting cytokines selected from the group consisting of granulocyte colony

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stimulating factor, granulocyte/macrophage colony stimulating factor, erythropoietin; and

(b) culturing said MNC in presence of copper chelator tetraethylenepentamine TEPA;

thereby expanding the population of said hematopoietic stem cell while inhibiting the differentiation of said HSC *ex vivo* in culture medium.

does not reasonably provide enablement for expanding any other population of hematopoietic stem cell or culturing mononuclear cells in presence of any other condition for proliferation or in presence of any other copper chelator. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

In determining whether Applicant's claims are enabled, it must be found that one of skill in the art at the time of invention by applicant would not have had to perform "undue experimentation" to make and/or use the invention claimed. Such a determination is not a simple factual consideration, but is a conclusion reached by weighing at least eight factors as set forth in In re Wands, 858 F.2d at 737, 8 USPQ 1400, 2d at 1404. Such factors are: (1) The breadth of the claims; (2) The nature of the invention; (3) The state of the art; (4) The level of one of ordinary skill in the art; (5) The level of predictability in the art; (6) The amount of direction and guidance provided by Applicant; (7) The existence of working examples; and (8) The quantity of experimentation needed to make and/or use the invention.

The office has analyzed the specification in direct accordance to the factors outlines in *In re Wands*. MPEP 2164.04 states: "[W]hile the analysis and conclusion of a

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lack of enablement are based on factors discussed in MPEP 2164.01(a) and the evidence as whole, it is not necessary to discuss each factor in written enablement rejection." These factors will be analyzed, in turn, to demonstrate that one of ordinary skill in the art would have had to perform "undue experimentation" to make and/or use the invention and therefore, applicant's claims are not enabled.

The aspects considered broad are: expanding population of hematopoietic stem cell from hematopoietic mononuclear cell, using any copper chelator for culturing mononuclear cell, using any condition for cell proliferation subsequently limiting to any cytokine or nutrient.

The specification fails to provide an enabling disclosure for the claimed invention because the specification fails to provide sufficient guidance as to how an artisan of skill would have practiced the claimed method of expanding hematopoietic stem cell by culturing MNC under any condition of proliferation in presence of any copper chelator. An artisan would have to carry out extensive experimentation to make and use the invention, and such experimentation would have been undue because art of expanding hematopoietic stem cell is still evolving several years after filing of instant application. In the instant case, specification fails to provide any guidance as to how the claimed method would have been practiced using MNC grown in presence of any combination of cytokines, nutrient or copper chelator. As will be shown below, broad aspects were not enabled for the claimed invention at the time of filing of this application because neither the specification nor the art of record taught sufficient guidance to practice the claimed

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invention over the full scope. For the purposes to be shown in the state of the prior art, the question of lack of enablement is discussed.

The specification discloses use of various agents in expanding hematopoietic stem cells present in the hematopoietic mononuclear cells fraction of a blood sample, without the use of a prior stem cells enrichment procedure (see page 4, para 5). Pages 25 provide brief description of the figures. The specification teaches *ex-vivo* expanded populations of hematopoietic stem cells, using hematopoietic mononuclear cells that comprise a major fraction of hematopoietic committed cells and a minor fraction of the hematopoietic stem and progenitor cells as a source of stem cells, without prior enrichment of the hematopoietic mononuclear cells for stem cells. The expanded populations of hematopoietic stem cells of the present invention can be used in variety of conditions including hematopoietic cell transplantation, in generation of stem cells suitable for genetic manipulations for cellular gene therapy (see page 26 of the specification). Pages 26-89 of the specification provide a detailed description of the invention, preferred embodiments and provide definition of terms. In addition, it is noted that specification asserts "hematopoietic mononuclear cells" refers to the entire repertoires of white blood cells present in a blood sample (see page 33, para 2 of the specification, while "hematopoietic stem cells" refers to pluripotent hematopoietic cells that, given the right growth conditions, may develop to any cell lineage present in blood (see page 34, para 2 of the specification). Rest of the specification provides specific examples describing the method of expanding an *ex vivo* population of hematopoietic stem cell by providing hematopoietic mononuclear cell and culturing in presence of

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cytokine and copper chelator. These broad disclosures do not demonstrate the information required by the Artisan to reasonably predict that HSC could be expanded *ex vivo* in presence of any cytokine or nutrient for any duration of time. The specification does not provide specific guidance commensurate with full scope of the claims.

Examples 1 of the specification describe that addition of TEPA chelator to non-purified MNC cultures, progressively increased the number of CD34+ cells, CD34+ colony-forming cells and CD34+CD38- cells, over a 12-week period (see example 1, pages 92-93; also see figure 1a, 1b and 2). It is noted that specification teaches that addition of Copper-TEPA chelator to MNC cultures markedly increases the number of CD34+ cells, and the number of CD34+CD38- cells, after an eight weeks incubation period (see example 2, page 94, Table 1).

As a first issue, the claims 201, 209-214, 238 and 239 embrace a method comprising expanding population of HSC in presence of any cytokine or copper chelator. Hofmeister et al (Bone Marrow Transplantation (2007) 39, 11-23) in a post filing art, while reviewing the state of *ex vivo* expansion of umbilical cord blood stem cells emphasize the importance of carefully choosing the *ex vivo* conditions that will expand the number of long-term reconstituting cell (LTRC) that requires evaluation of characteristics of the expanded cells and their interaction with both the soluble and insoluble components of *ex vivo* culture. Hofmeister states, "Isolating cells to expand based on only their surface protein expression is likely to include undifferentiated and mature cells. Hofmeister cite references to indicate that "The surface phenotype can change depending on the activation status of the precursor cells and does not provide

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information on the functional ability of the cells *in vivo*. Although CD34 expression has been the most commonly selected surface marker for *ex vivo* expansion, CD34 is often expressed on more differentiated cells and large animal models suggest that CD34+ cells are not the cells primarily involved in marrow reconstitution, and following *ex vivo* culture, dissociation between CD34+CD38- cell expansion and SCID-repopulating capacity has been observed". It is noted that Hofmeister et al describe that preliminary data suggests that as umbilical cord blood units are made up of progenitor cells that possess both a CD34+ and a CD34- phenotype, isolating a more primitive marker CD133+ may identify cells that are less mature than those that express CD34". This is further supported by studies conducted by Gallacher et al (Blood, 2000, 95(9): 2813-20) that indicated that human hematopoietic stem cell properties can be found among cells lacking CD34 and lineage commitment markers (CD34(-)Lin(-)). Gallacher et al used cell surface markers AC133 and CD7, and isolated CD34(-)CD38(-)Lin(-) and CD34(+)CD38(-)Lin(-) cell sub fractions from human cord blood that resulted in identification of a population of AC133(+)CD7(-) cells that are highly enriched for progenitor activity at a frequency equivalent to purified fractions of CD34(+) stem cells. It is noted that these cells are the only subset among the CD34(-)CD38(-)Lin(-) population capable of giving rise to CD34(+) cells in defined liquid cultures (see abstract and Figure 3 and 4). In the instant case, specification provides guidance with short-term MNC cell cultures supplemented with TEPA in presence of cytokines, enabled tremendous expansion of CD34+ cells and stem/early progenitor cells (CD34+38-) as compared with minimal expansion of these cells obtained in MNC cultures treated only

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with cytokines. It is emphasized that specification provide no guidance that instant method would expand the entire population of CD34- cells, particularly since specification merely contemplates that the results may also suggest that in addition to the regulatory effect on CD34+ cells and its early subsets, the chelator may also enable ex-vivo expansion a small subset of cells that are not co-purified with the CD34+ cell fraction. This subset of cells, which is probably in nature CD34-, may support superior expansion of CD34+ cells and its subsets during the extended long-term cultures (See page 93, last para, bridging to page 94). Thus, cited art indicates that neither prior art nor specification identified a subset of CD34- population of cells capable of providing CD34+ cell. In addition, prior art also teaches CD133+ as better marker that would also embrace cells that are less mature than those that express CD34. Therefore, an artisan would have to perform undue experimentation to further characterize different population of cells that are less mature than those that express CD34 in presence of different combination of cytokine and copper chelator to conclusively establish that instant method would result in *ex vivo* expansion of HSC commensurate with full scope of the claim .

As a second issue, claims 201, 209-211, 213, 238 and 239 embrace expanding HSC in presence of any condition that allows cell proliferation of hematopoietic mononuclear cells (MNC). The specification contemplates providing the stem cells with the conditions for ex-vivo cell proliferation by providing the cells with nutrients and cytokines that may be early and late acting cytokines. Prior to instant invention, Murray et al (Exp Hematol. 1999; 27(6): 1019-28) disclose the importance of various

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combinations of cytokines that have profoundly different effects on inhibition of apoptosis and stimulation of self-renewal division of hematopoietic stem cells (HSC) in short-term, ex vivo culture. Murray et al emphasize that the balance of cell cycling, division history, differentiation, and apoptosis of CD34⁺ cells determines the net number of HSC produced in ex vivo cultures (see abstract). It is noted that single cytokines thrombopoietin (TPO), flt3 ligand (FL), and c-kit ligand (KL) each failed to increase the number of CD34⁺Thy-1⁺ cells, however, cultures including TPO, FL or TPO, KL gave the increase of CD34⁺Thy-1⁺ cell number. It is emphasized that considering the level of variation among the mobilized peripheral blood (MPB) samples, there was no significant difference among such cultures, which all resulted in maintenance or a small increase (1.2- to 1.5-fold) of CD34⁺Thy-1⁺ cell number. The cited art clearly indicate the importance of right balance to cytokine and nutrient combination for optimal expansion of HSC as contemplated by the specification. In the instant case, specification exemplified ex-vivo expansion of hematopoietic stem cells from the MNC in a media that was supplemented with tetraethylpantamine (TEPA) chelator and with human recombinant Thrombopoietin (TPO), interleukin 6 (IL-6), FLT-3 ligand, and a stem cell factor (SCF), which is interchangeable with IL-3 (see example 1). It is emphasized that this does not teach how to extrapolate data to include any combination of cytokine that minimally allows the proliferation of HSC and still facilitates the expansion of HSC when grown in presence any copper chelator. It is apparent from the cited art that instant specification does not provide guidance that any combination of cytokine would necessarily expand the ex vivo of population of HSC as broadly recited in claims 201

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209-211, 213, 238 and 239 and therefore these claims are not enabled commensurate with the full scope of the claims.

As a final issue, the claims 201, 209-214, 238 and 239 embrace a method uses culturing mononuclear cells in presence of at least one copper chelator. The specification has exemplified only culture medium supplement with chelator tetraethylpantamine (TEPA) in presence of right combination of cytokine result in effective expansion of HSC from the MNC. Peled et al (Exp Hematol. 2004; 32(6): 547-55) show that the only low-molecular-weight linear polyamine Cu chelator TEPA at a concentration that moderately reduced cell Cu content (by 20–30%) enabled extensive *ex vivo* expansion of CD34⁺ cells in cultures supplemented with early-acting cytokines (see page 552). It is emphasized that neither prior nor specification provide any guidance that instant method could be practiced using any copper chelator. In fact, specification acknowledges that while reducing the present invention to practice, it was surprisingly and unexpectedly found that molecules such as copper chelator repress differentiation and stimulate and prolong proliferation of hematopoietic stem cells (see page 28, lines 26-30). In addition, prior studies have indicated that effect of copper chelator in effecting cellular function is contradictory to one disclosed in the instant application. Percival et al (Am J Clin Nutr. 1998; 67(5 Suppl): 1064S-1068S) while reviewing the role of copper hypothesize that if copper is essential for differentiation, then chelation of copper with TEPA should prevent the cells from differentiating. Percival et al indicated that cells incubated with TEPA and retinoic acid produced the same amount of superoxide anion as did the cells with retinoic acid, indicating that

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differentiation occurred suggesting more work may be required to study the exact role of genus of copper chelator in expansion of HSC (See page 1066S, col. 2, para. 2). An artisan would have to carry out extensive experimentation to make use the invention, and such experimentation would have been undue because of the neither art nor specification provide any guidance commensurate with full scope of the claims as to how the claimed method would have been practiced for expanding HSC using any copper chelator.

Because of the art, as shown above, does not disclose how hematopoietic stem cells could be expanded *ex vivo* by culturing MNC in presence of any cytokine, nutrient or copper chelator. Artisan could not predict, in the absence of proof to the contrary, that such a method of expanding hematopoietic stem cell would be successful. An artisan would have to carry out extensive experimentation to make use the invention, and such experimentation would have been undue because of the art of expanding HSC *ex vivo* in presence of any cytokine or nutrient for any duration was unpredictable and specification fails to provide any guidance as to how the claimed method would have been practiced commensurate with full scope of the claim.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

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Claims 201, 209-214, 238 and 239 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 201 is vague and indefinite because it recites term "substantially inhibiting differentiation" which is subject to variable interpretation depending on artisan. Since, different conditions to different cells will have different inhibitory capacity and thus what may be substantial in one cell type and for one condition may not be substantial in another. Therefore, meets and bound of term substantially inhibiting differentiation cannot be determined. The term "substantially" in claim 201 is a relative term, which renders the claim indefinite. The term "substantially" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. In the instant case, it is unclear "substantially inhibiting differentiation" is relative to what? Claims 209-214, 238 and 239 directly on indirectly depend on claim 201. Appropriate correction is required.

Claim 239 recites the limitation "hematopoietic cell". There is insufficient antecedent basis for this limitation in the claim. It is unclear whether claim 239 refers to HSC or hematopoietic mononuclear cell of claim 201. Appropriate correction is required.

Claim Rejections - 35 USC § 102

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The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

Claims 201, 209-214 and 238-239 are rejected under 35 U.S.C. 102(a) as being anticipated by Peled et al (Blood, November 16 2002; Vol. 100, No. 11, pp. abstract No. 4076).

It is noted that the reference of Peled et al. shares co-authorship with Treves, Avraham of the instant application. However, the reference of Peled et al. was also written by Iudin, Dimitry; Gluckman, Elina; Adi, Sophie; Hasson, Nira, who are not listed as inventors of the instant invention. Therefore, the reference of Peled et al was written by a different inventive entity than that of the instant invention.

Peled et al teach a method showing presence of polyamine copper chelators, in a hematopoietic mononuclear cell cultures for three weeks, markedly potentiated the long-term expansion of CD34+ hematopoietic stem/progenitor cells and long-term culture colony forming cells (LTC-CFUc). Peled et al show that MNC were cultured in tissue culture bags, in serum containing medium, 50ng/ml FLT3, IL-6, TPO and SCF, with or without copper chelator (TEPA; 5-10uM). The results show short-term treatment of MNC cultures with a polyamine copper chelator modulated CD34+ cells and their early subsets response to intrinsic or extrinsic signals resulting in further expansion of these progenitors during the long-term cultures. Peled contemplated that copper chelator used in this manner could be used for ex vivo expansion from MNC

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cultures with no need for pre enrichment for CD34+ cells (see the entire abstract). The presence of CD34+ and a minor portion of CD34- cells are inherent in the population of cells disclosed by Peled et al.

Accordingly, the invention of claims 201, 209-214, 235 and 238 are anticipated by Peled et al because steps recited in the claims are the same as those taught by the cited arts.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 201, 209-214, 238 and 239 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sandstrom et al (Blood. 1995; 86(3): 958-70, IDS) and Peled et al (WO99/40783, 8/19/1999, IDS).

Prior to instant invention, Sandstrom et al teach a method of ex vivo expansion of peripheral blood mononuclear cells (MNCs), cultured both directly and after selection for CD34+ cells and also compared in static and continuously perfused cultures containing different cytokines such as interleukin (IL)-3, IL-6, granulocyte colony-stimulating factor (G-CSF), and stem cell factor (SCF). Sandstrom discloses that peripheral blood (PB) and BM MNCs contain many different cell types at various stages of maturation.

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Sandstrom et al describe the advantage of using CD34+ enriched population but compares the total and progenitor cell production of MNC and CD34+ cell cultures (See page 958, col. 2). It is noted that Sandstrom et al examined how CD34+ selection and/or perfusion affect the performance of PB MNC cultures supplemented with serum and combination of cytokines (see page 959, col. 1). It is noted that Sandstrom et al teach that either cultures inoculated with MNCs or CD34+ cells produced cells that were remarkably similar after 10 days of culture. In addition, Sandstrom et al discloses It is noted that total numbers of cells, CFU-GM, and LTC-IC that could be obtained from perfusion culture of a PB sample cultured as MNCs were greater than those that could be obtained from the same sample selected and cultured as CD34+ cells (see page 964, column 2 and Table 8). However, Sandstrom differed from claimed invention by not comparing the culture of MNC or CD34+ cells in presence of a copper chelator.

Peled et al teach a method of expanding a population of cells including HSC obtained from neonatal umbilical cord blood, while at the same time inhibiting differentiation of the cells. Peled et al teach culturing cells in presence of early and late acting cytokines and, at the same time, for reducing a capacity of the cells in utilizing transition metals such as copper chelator tetraethylenepentamine (TEPA) (See claims 1-17). It is noted that Peled et al also looked the effect of TEPA on the maturation of hematopoietic cells in a two-phase liquid culture procedure wherein peripheral blood mononuclear cells are first incubated in the presence of early growth factors and then in second phase, these factors are replaced by the erythropoietin in presence of copper chelator TEPA (see page 25). The results suggested that TEPA inhibited the erythroid

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differentiation, but did not significantly affect the proliferation ability of the progenitor cells (see figure 5 and page 25). Peled et al also show that presence of TEPA sustains long-term cultures of HSC in a CD34+ enriched population of cells by inhibiting/delaying cellular differentiation through chelation of copper (see example 2 and 3). Although, Peled et al taught method steps same as one disclosed in the instant claims but differed from claimed invention by disclosing expansion of HSC from a CD34+ enriched population and not from a unselected mononuclear cell population as claimed in the instant invention.

Accordingly, in view of the teachings of Sandstrom and Peled, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method of taught by Sandstrom by culturing MNC directly and after selection for CD34+ cells in presence of a copper chelator such as TEPA with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as it was art recognized goal to *ex-vivo* expand cells that include HSC and more differentiated progenitor cells in order to optimize short-term recovery and long-term restoration of hematopoiesis. Sandstrom emphasized the importance of expansion of all progenitor cells, especially those committed to the neutrophilic and megakaryocytic lineages, concomitant with expansion of stem cells in order to decrease the extent and duration of cytopenias after transplantation (see page 967, col. 2 bridging to page 968, col. 1). In addition, Peled had already disclosed that CD34+ cell cultures with early-acting cytokines and TEPA, it is possible to maintain long term cultures (LTC) without the support of stroma (see

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example 2). Although Peled et al did not use mononuclear cell, he used peripheral blood mononuclear cells in a two-phase liquid culture to show the potential of TEPA in inhibiting erythroid differentiation (supra). Sandstrom sought to examine how CD34+ selection affect the performance of MNC cultures supplemented with different combination of cytokines. Therefore, given that copper chelator such as TEPA was available for use to expand CD34+ enriched population of HSC as per the teachings of Peled, it would have been obvious to an artisan to use copper chelator such as TEPA in the unselected or CD34+ selected cells taught by Sandstrom. The skilled artisan would be motivated to modify the method of Sandstrom in order to compare the expansion potential and to determine the role of TEPA on expansion of cells that are committed to the neutrophilic and megakaryocytic lineages concomitant with expansion of stem cells to reduce the extent and duration of cytopenias after transplantation. In addition, such a direct *ex vivo* expansion from MNC would have also resulted in fewer steps to quickly obtain HSC for various transplantation purposes. It is noted that obtaining MNC from different source was routine practice in the art for expanding HSC and the skilled Artisan would have motivated to optimize method to obtain MNC from different source for expansion of HSC (see MPEP 2144.04).

One who would practiced the invention would have had reasonable expectation of success because Sandstrom had already described a method of *ex vivo* expansion of peripheral blood mononuclear cells (MNCs), cultured both directly and after selection for CD34+ cells in presence of cytokine. Peled had already described use of copper chelator such as TEPA could be used for inhibiting differentiation of MNC or expanding

CD34+ cells. Thus, it would have only required routine experimentation to modify the method disclosed by Sandstrom to also culture cell in presence of TEPA as required by instant invention.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No Claims allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272- 4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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